PATENT
Customer No. 22,852
Attorney Docket No. 06478.1507-00000

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| In re Application of: | |
| Reinhard **BOLLI** *et al.* | Group Art Unit: 1644 |
| Application No.: 10/579,357 | Examiner: Kim YUNSOO |
| Filed: May 16, 2006 | |
| | Confirmation No.: 2138 |
| For: IMMUNOGLOBULIN PREPARATIONS HAVING INCREASED STABILITY | |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

**DECLARATION UNDER 37 C.F.R. § 1.132**

I, Reinhard Bolli, do hereby make the following declaration:

1. I am a Swiss citizen, residing at Bellevuestr. 14, CH-3073 Gümligen, Switzerland.

2. I have been awarded a degree in Chemistry from the University of Basel and a Doctoral degree (Ph.D.) in phil. Nat. (Biochemistry) from the University of Bern (Switzerland).

3. I have been employed by CSL Behring since 01.10.1988 and am presently a senior manager of the Biochemistry department R&D at CSL Behring. During my employment at CSL Behring, I have been engaged in research and development regarding the investigation of plasma proteins including immunoglobulins.

4. I am an inventor of the subject matter in Application No. 10/579,357

5. I have read and understand Application No. 10/579,357, including the claims as amended in the response filed herewith. For instance, I understand that independent claim 1, as amended, now recites a stable immunoglobulin preparation, wherein the preparation comprises proline and wherein the preparation has a pH of 4.2 to 5.4 and wherein the preparation does not comprise nicotinamide. I also understand that independent claim 8, as amended, recites a stable immunoglobulin preparation, wherein the preparation comprises proline and has a pH of 4.2 to 5.4, and wherein the final concentration of proline is between 0.2 to 0.4 M.

6. I have read and understand the specification and claims of U.S. Patent No. 5,871,736 ("the '736 patent") directed to a immunoglobulin preparation.

7. The '736 patent teaches that "preferred stabilizers are compositions comprising nicotinamide together with one or more of the . . . amino acids or their derivatives." See the '736 patent, col. 4, lines 28-30. In addition, as shown in table 2 and table 5 of the '736 patent, proline was used in conjunction with nicotinamide and only in concentrations of up to 0.2 M. Proline was never disclosed, taught or suggested as sufficient to stabilize the composition in the absence of nicotinamide.

8. However, contrary to the teachings of the '736 patent, I have discovered, unexpectedly, that the use of proline alone and without nicotinamide is beneficial for immunoglobulin preparations. I also found, unexpectedly and apart from the teaching of the '736 patent, that proline at a final concentration between 0.2 to 0.4 M led to a decreased level of aggregate formation and coloring of immuoglobuline preparations.

9. In order to demonstrate the difference between the immunoglobulin preparations disclosed in the '736 patent and the present application, I prepared and

tested several immunoglobulin solutions, described below and shown in Tables 1-2 and Figures 1-3.

**Comparative testing of IgG solutions with or without the addition of nicotinamide**

10. CSL Behring purified IgG from pooled human plasma by cold ethanol and octanoic acid fractionation followed by anion exchange chromatography and concentrated IgG to approximately 100 mg/ml (10%) by ultrafiltration using large scale state of the art procedures. In my laboratory the 10% IgG solutions were formulated with or without L-proline and/or nicotinamide at concentrations of 0, 125, 250, 350 and 500 mmol/L at a pH of 4.8 ± 0.2 (See Table 1 below). These different formulations were then incubated at 40°C in the dark for up to 30 days. At day 0 and day 30 of the incubation, in my laboratory size exclusion HPLC with a TSK 3000SW column was used to analyze the percentage of aggregates in the different IgG formulations and UV/VIS photometry to measure the yellowish colouring (i.e. absorbance at 350nm) of the solutions. The results are shown in Table 1, Figure 1, and Figure 2.

**Table 1:** IgG-solutions (100mg/ml, pH 4.8 ± 0.2) were incubated at 40°C and analysed after 30 days storage.

| Formulation | | Aggregates (%) | | Absorbance at 350nm | |
|---|---|---|---|---|---|
| Proline (mmol/L) | Nicotinamide (mmol/L) | Day 0 | 30d | day 0 | 30d |
| 0 | 0 | < 0.1 | 1.3 | 0.052 | 0.072 |
| 125 | 0 | < 0.1 | 1.1 | 0.065 | 0.069 |
| 0 | 125 | < 0.1 | 3.9 | 0.073 | 0.094 |
| 250 | 0 | < 0.1 | 0.9 | 0.061 | 0.070 |
| 0 | 250 | < 0.1 | 10.3 | 0.084 | 0.116 |
| 350 | 0 | < 0.1 | 0.7 | 0.069 | 0.066 |
| 0 | 350 | < 0.1 | 19.5 | 0.087 | 0.157 |
| 500 | 0 | < 0.1 | 0.6 | 0.086 | 0.064 |
| 0 | 500 | 0.4 | 37.2 | 0.082 | 0.271 |
| 125 | 125 | < 0.1 | 3.0 | 0.075 | 0.090 |
| 250 | 250 | < 0.1 | 7.7 | 0.085 | 0.105 |
| 350 | 350 | < 0.1 | 13.7 | 0.097 | 0.129 |
| 500 | 500 | 0.2 | 26.1 | 0.102 | 0.186 |

**Figure 1**: Aggregate formation of 10% IgG solutions after storage at 40°C for 30 days.

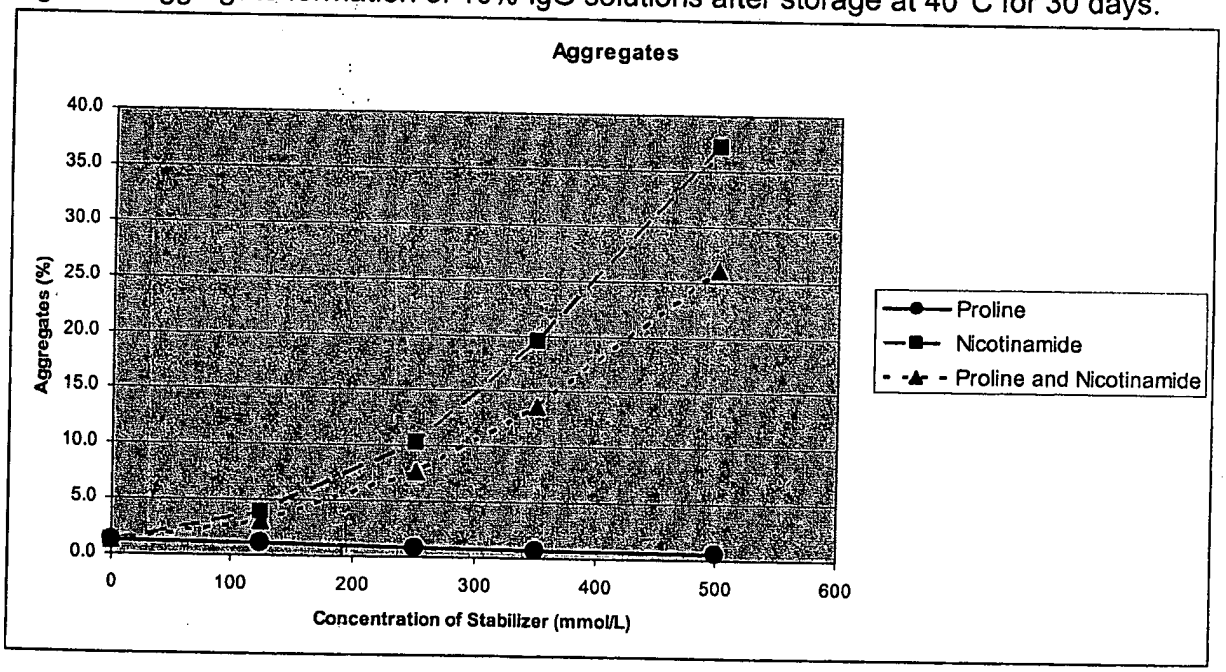
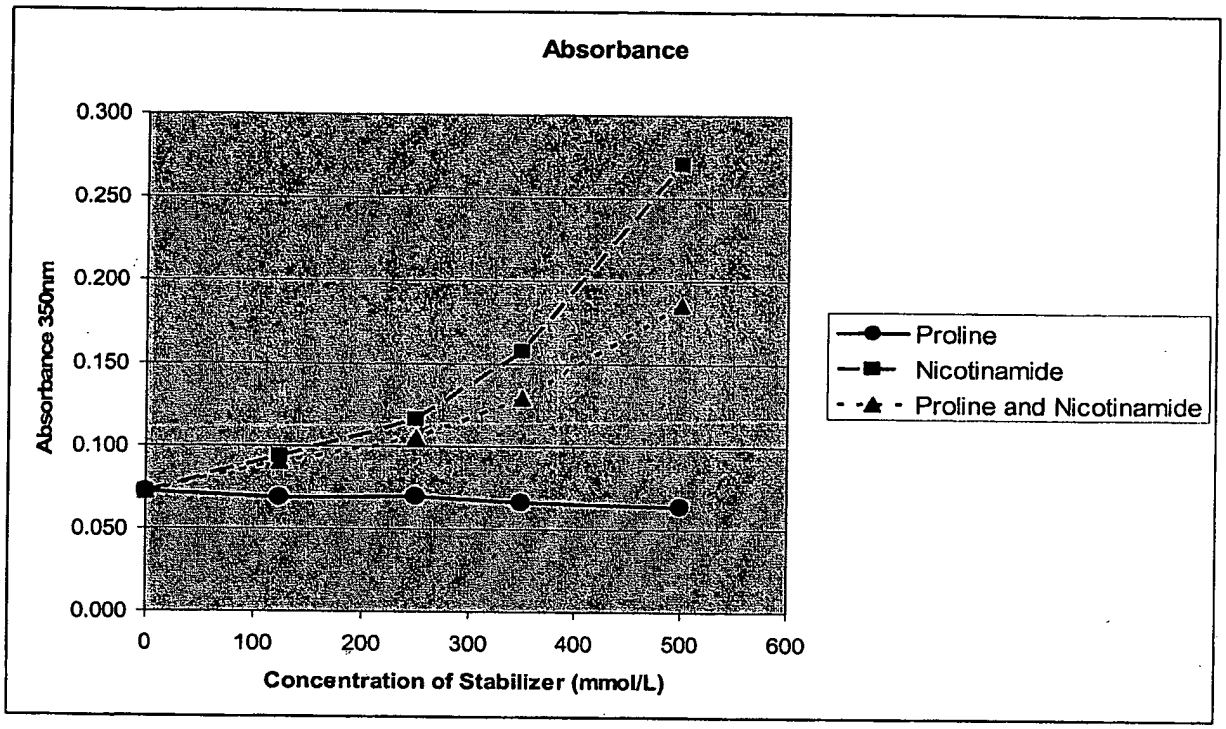


**Figure 2:** Absorbance at 350 nm (yellowish colouring) of 10% IgG solutions after storage at 40°C for 30 days.




11. The results showed that 10% IgG formulations with proline alone had lower percentage of aggregates and less degree of coloring as compared to the formulations with nicotinamide alone or the formulations with both proline and nicotinamide. Based on my education and experience, these results are unexpected in view of the teachings of the '736 patent, which taught using proline and nicotinamide.

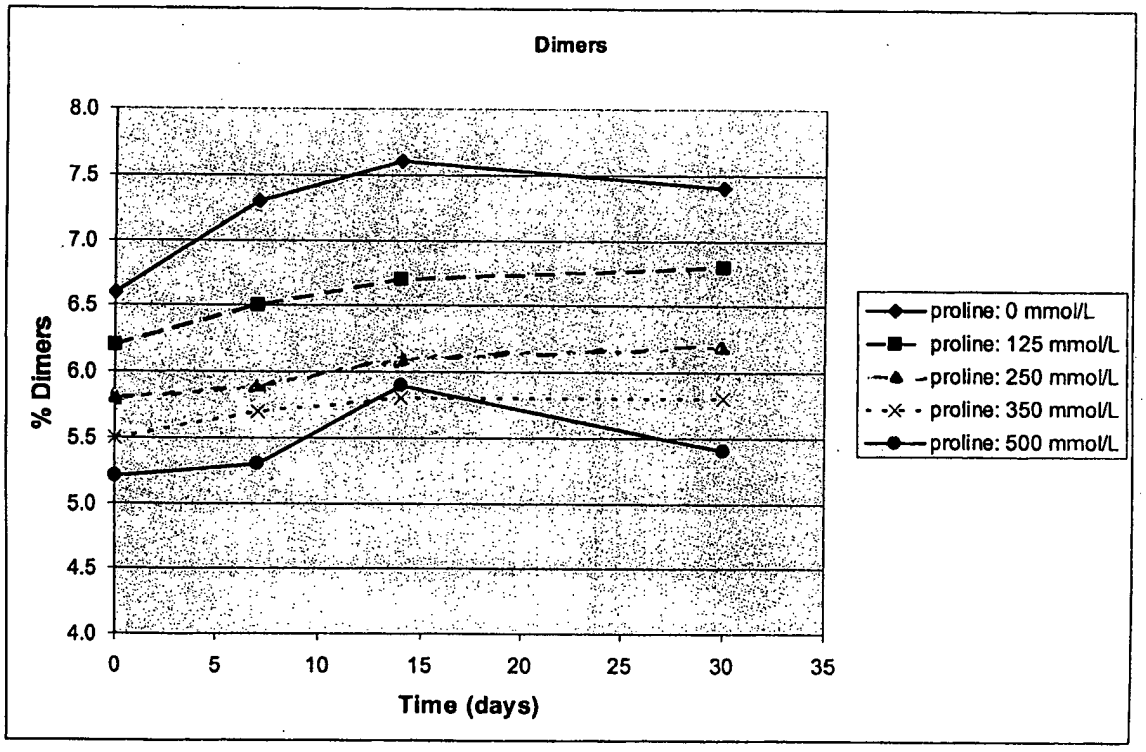
**Dimer formation of IgG solutions formulated with proline at different final concentrations**

12. 10% IgG solutions were prepared as described above and formulated with 0,125, 250, 350 and 500 mmol/L proline at a pH of 4.8 ± 0.2. The formulations were then incubated at 40°C in the dark. After indicated times of incubation, my laboratory used size exclusion HPLC with a TSK 3000SW column to measure the content of IgG dimers in the solution. The results are shown in Table 2 and Figure 3.

**Table 2:** IgG-dimer formation in 10% IgG solutions during storage at 40°C

| Proline (mmol/L) | Dimer content (%) | | | |
|---|---|---|---|---|
| | Day 0 | Day 7 | Day 14 | Day30 |
| 0 | 6.6 | 7.3 | 7.6 | 7.4 |
| 125 | 6.2 | 6.5 | 6.7 | 6.8 |
| 250 | 5.8 | 5.9 | 6.1 | 6.2 |
| 350 | 5.5 | 5.7 | 5.8 | 5.8 |
| 500 | 5.2 | 5.3 | 5.9 | 5.4 |

**Figure 3:** Dimer formation in 10% IgG solutions during storage at 40°C for 30 days



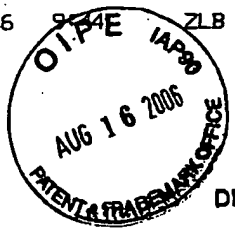

13. The results indicated that the dimer content or the dimer formation was significantly reduced in IgG-solutions formulated with proline alone. A satisfying

reduction of dimer content was achieved between 200mM and 400mM proline. However, proline concentration at 500 mM did not seem to result in consistent and uniform reduction of dimer formation over time. In addition, to the extent that the '736 patent merely discloses a proline concentration of up to 200 mM, I believe a skilled artisan would have no reason nor motivation to increase the proline concentration beyond 200 mM because it would increase the cost of the preparation and the osmolarity of the solution, both of which could lead to undesirable outcomes for clinical applications. Accordingly, I believe that the claimed invention is novel and unexpected in view of the concentration range suggested by the '736 patent.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 28.01.09 By: R. Bolli

Reinhard Bolli






# DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

## IMMUNOGLOBULIN PREPARATIONS HAVING INCREASED STABILITY

the specification of which
☐ is attached and/or
☒ was filed on May 16, 2006 as U.S. Application Serial No. 10/579,357 and amended on May 16, 2006 and/or
☒ was filed on November 17, 2004 as PCT International Application No. PCT/EP2004/013022 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States, listed below and have also identified below, any foreign application(s) for patent or inventor's certificate, or any PCT international application(s) having a filing date before that of the application(s) of which priority is claimed:

| Country | Application Number | Date of Filing | Priority Claimed Under 35 U.S.C. 119 |
|---|---|---|---|
| EUROPE | 03026539.1 | November 18, 2003 | ☒ YES ☐ NO |
| | | | ☐ YES ☐ NO |

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

| Application Number | Date of Filing |
|---|---|
| | |
| | |

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT international application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

| Application Number | Date of Filing | Status (Patented, Pending, Abandoned) |
|---|---|---|
| | | |

I hereby appoint the following attorney and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

**FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., CUSTOMER NUMBER 22,852.**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

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# MOLECULAR BIOLOGY OF
# THE CELL

## SECOND EDITION

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Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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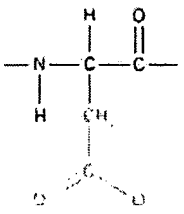
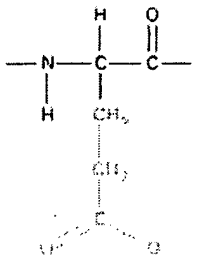
## ACIDIC SIDE CHAINS

**aspartic acid**
(Asp, or D)



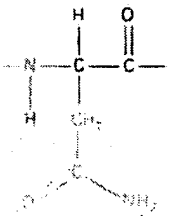
**glutamic acid**
(Glu, or E)



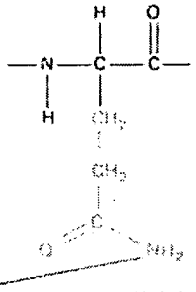
Amino acids with uncharged polar side chains are relatively hydrophilic and are usually on the outside of proteins, while the side chains on nonpolar amino acids tend to cluster together on the inside. Amino acids with basic and acidic side chains are very polar and they are nearly always found on the outside of protein molecules.

## UNCHARGED POLAR SIDE CHAINS

**asparagine**
(Asn, or N)



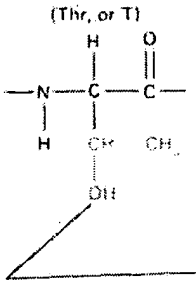
**glutamine**
(Gln, or Q)



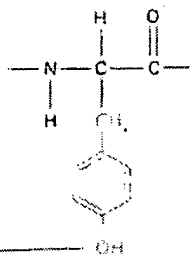
Although the amide N is not charged at neutral pH, it is polar.

**serine**
(Ser, or S)

**threonine**
(Thr, or T)



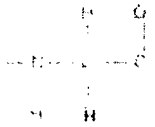
**tyrosine**
(Tyr, or Y)



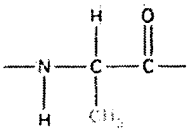
OH group is polar.

## NONPOLAR SIDE CHAINS

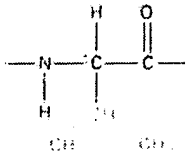
**glycine**



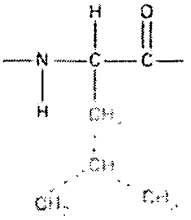
**alanine**
(Ala, or A)



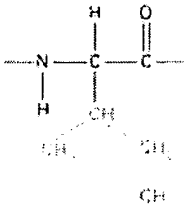
**valine**
(Val, or V)



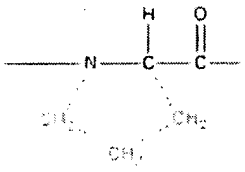
**leucine**
(Leu, or L)



**isoleucine**
(Ileu, or I)

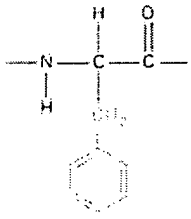


**proline**
(Pro, or P)

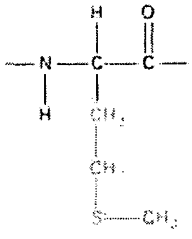


(actually an imino acid)

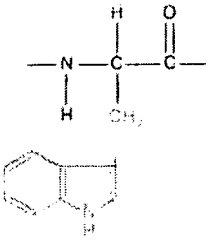
**phenylalanine**
(Phe, or F)



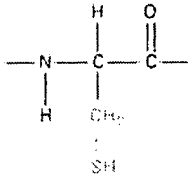
**methionine**
(Met, or M)



**tryptophan**
(Trp, or W)



**cysteine**
(Cys, or C)



Paired cysteines allow disulfide bonds to form in proteins.